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Clustering of HCV coinfections on HIV phylogeny indicates domestic and sexual transmission of HCV

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Abstract

Background: HCV coinfection remains a major cause of morbidity and mortality among HIV-infected individuals and its incidence has increased dramatically in HIV-infected men who have sex with men(MSM).

Methods: Hepatitis C virus (HCV) coinfection in the Swiss HIV Cohort Study(SHCS) was studied by combining clinical data with HIV-1 *pol*-sequences from the SHCS Drug Resistance Database(DRDB). We inferred maximum-likelihood phylogenetic trees, determined Swiss HIV-transmission pairs as monophyletic patient pairs, and then considered the distribution of HCV on those pairs.

Results: Among the 9748 patients in the SHCS-DRDB with known HCV status, 2768(28%) were HCV-positive. Focusing on subtype B(7644 patients), we identified 1555 potential HIV-1 transmission pairs. There, we found that, even after controlling for transmission group, calendar year, age and sex, the odds for an HCV coinfection were increased by an odds ratio (OR) of 3.2 [95% confidence interval (CI) 2.2, 4.7) if a patient clustered with another HCV-positive case. This strong association persisted if transmission groups of

intravenous drug users (IDUs), MSMs and heterosexuals (HETs) were considered separately(in all cases OR >2). Finally we found that HCV incidence was increased by a hazard ratio of 2.1 (1.1, 3.8) for individuals paired with an HCV-positive partner.
Conclusions: Patients whose HIV virus is closely related to the HIV virus of HIV/HCV-coinfected patients have a higher risk for carrying or acquiring HCV themselves. This indicates the occurrence of domestic and sexual HCV transmission and allows the identification of patients with a high HCV-infection risk.

Key words: HIV-HCV coinfection, molecular epidemiology, genotypic resistance testing, sexual transmission of HCV

Key Messages

- Location of patients on HIV phylogeny is predictive of HCV incidence and prevalence (even after controlling for transmission group).
- This effect is strong in MSMs, supporting domestic sexual transmission of HCV.
- This approach represents a novel method for identifying HIV-infected individuals with a high risk for HCV coinfections.

Introduction

Hepatitis C virus (HCV) is one of the major causes of chronic liver disease. Overall 3% of the world population are infected with HCV.[1] Of those infected, 20–50% develop liver cirrhosis and 5% hepatocellular carcinoma.[2,3] The epidemics of HIV and HCV interact in multiple ways. Accordingly, a substantial fraction of HIV-infected individuals also carry HCV. Both viruses can be transmitted parenterally by contaminated blood (needlesticks, blood transfusions etc.) or sexually. However, the efficiency of these transmission routes differs strongly between the two viruses. HCV is more efficiently transmitted by needlesticks than HIV(approximately by a factor of 10)[4] whereas its transmission rate upon sexual contact is much lower. Several studies have even questioned the epidemiological importance of sexual transmission for HCV.[5] Accordingly, HIV-HCV coinfections in Western Europe occurred, until recently, almost exclusively in intravenous drug users (IDU). In recent years, however, HCV incidence has increased dramatically in HIV-infected men who have sex with men (MSM).[6–10] The mechanisms behind this development are unclear (e.g. increase in traumatic sexual practices,[9] decreased condom use[11] etc.) and non-injecting drug consumption may also constitute a transmission route.[9] Nevertheless, the accumulated evidence[6–10] indicates an increased role of sexual transmission for HCV in HIV-infected patients.

Despite the shared transmission mechanisms, it is unclear to what extent the two viruses also share entire transmission networks. The Swiss HIV Cohort Study (SHCS) is a unique research tool for studying how the HIV and HCV epidemics interact. First, it is unique in terms of its representativeness of the HIV epidemic in an entire country. Specifically, it includes an estimated 45% of all HIV-infected patients in Switzerland (since 1988) and represents all major risk groups (MSM, IDU, HET) and geographical regions in Switzerland.[12] Second, since 1998, HCV-negative individuals in the SHCS have been screened at least every 2 years for HCV. This allows a detailed estimation of HCV incidence and prevalence in the HIV-infected population in Switzerland. Finally, the SHCS is linked to the SHCS-Drug-Resistance Database (SHCS-DRDB) which contains >15 000 sequences from >10 000 patients in the SHCS. This allows combining molecular epidemiology approaches with the traditional epidemiological analysis of disease spread.

Molecular epidemiology approaches have made important contributions to understanding the spread of infectious diseases such as HIV-1.[13–19] The molecular epidemiology of HIV-1 has been facilitated by the fact that HIV-1 sequences are routinely generated in the context of genotypic drug resistance tests. By contrast, only few HCV sequences are available for most HCV epidemics, which limits classical molecular epidemiology analyses. Here, we study the

interaction between the spreads of HIV and HCV in Switzerland by analysing the distribution of HCV cases on the population-level phylogeny of HIV. This population-level phylogeny describes the clustering of HIV sequences from different patients and characterizes thereby the transmission chains of HIV. Accordingly, we expect the degree of clustering of HCV on this phylogeny to be a measure of the intensity of the interaction between the HIV and HCV epidemics.

Methods

We explored the spread of HCV among HIV-infected individuals in Switzerland by combining clinical and demographic data from the SHCS with HIV-1 nucleotide sequences (partial *pol* gene: entire protease and partial reverse transcriptase) from the SHCS-DRDB. The SHCS is a nationwide, prospective, clinic-based cohort study with continuous enrolment and semi-annual study visits (currently over 17 000 patients enrolled).[12,20,21] The SHCS has been approved by ethical committees of all participating institutions and written informed consent has been obtained from participants. The SHCS-DRDB includes the genotypic drug resistance test results generated in the context of the SHCS: all 4 laboratories allowed to perform resistance testing in Switzerland contribute all their resistance test results to the SHCS-DRDB. Furthermore, retrospective sequencing was performed systematically from the sample repository to obtain a sequence for all patients since 1996. In total, the SHCS-DRDB contains 15 626 sequences from 10 139 patients. HCV status was known for 9748/10 139 patients. From these, we used for the phylogenetic part of the analysis the earliest sequence of each patient infected with HIV-1 subtype B (7644 sequences; download: 26 November 2012). We pooled these sequences with all corresponding available subtype B sequences from the Los-Alamos sequence database (36 227 sequences, criteria for selecting these sequences: entire protease had to be available and sequence length had to exceed 1000 nucleotides; download: 26 November 2012).

From this pooled dataset, we inferred maximum-likelihood phylogenetic trees with FastTree 2[22] (assuming the GTR model of molecular evolution). Codons, where major drug resistance mutations occur (codons 30, 32, 33, 46, 47, 48, 50, 54, 76, 82, 84, 88 and 90 in the PR, and 41, 62, 65, 67, 69, 70, 74, 75, 77, 100, 103, 106, 108, 115, 116, 151, 181, 184, 188, 190, 210, 215, 219, 225 and 236 in the RT) were excluded from the analysis in order to avoid distortion by convergent evolution. FastTree was used to infer the phylogeny because it is considerably faster than RaxML[23] and has been shown to exhibit only 'little and (in some cases no) degradation in tree accuracy, as compared to RAxML'[24]. We determined on these trees potential Swiss HIV-transmission pairs as monophyletic pairs of Swiss patients (see Figure 1 for a schematic representation; see also supplementary material for an extension of this approach to larger clusters, available as Supplementary data at *IJE* online). Then we analysed the distribution of HCV cases on the tree and the potential transmission pairs using univariable and multivariable logistic regression models.

Positive HCV tests were determined by either positive HCV serology[enzyme-linked immunosorbent assay (ELISA) confirmed by immunoblotting] or by detectable

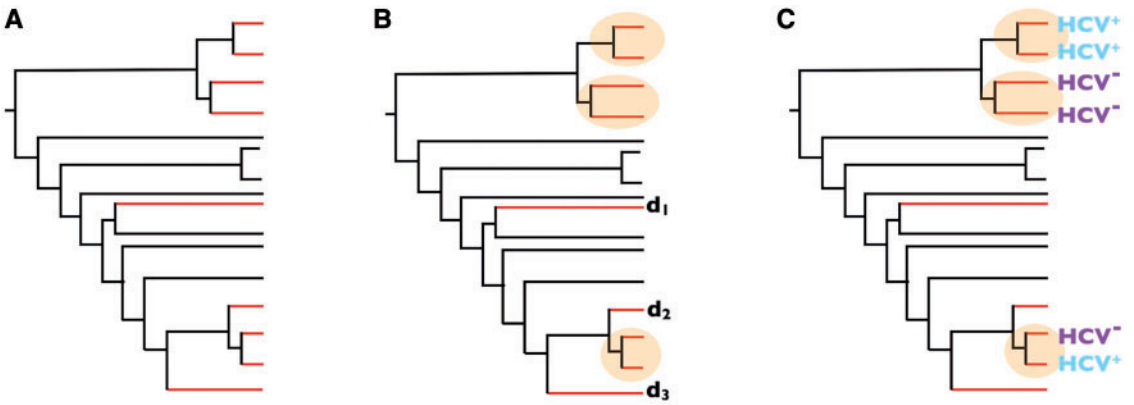

Figure 1. Schematic representation of the rationale of the phylogenetic analysis. **(A)** In the first step, the Swiss sequences from the SHCS-DRDB are pooled with all available foreign sequences from the Los-Alamos Sequence Database and a phylogenetic tree is inferred (red tips: Swiss sequences; black tips: non-Swiss sequences; note that in the real tree, most sequences are non-Swiss). **(B)** In the second step, Swiss transmission pairs are inferred as monophyletic pairs of Swiss sequences (indicated by orange ellipses). Note that in this analysis, we discarded sequences that belong to pairs with foreign sequences (such as sequence d_1) and sequences that are not part of monophyletic pairs (such as d_2 and d_3). In the supplementary material (available as Supplementary data at *IJE* online) we present an analysis that also includes such sequences discarded in the main analysis. (**C**) In the third step, we consider the distribution of HCV states over the transmission pairs. Three constellations are possible: HCV-sero-concordant and -positive (upper pair), HCV-sero-concordant and -negative (middle pair) and HCV-sero-discordant (lower pair).

HCV RNA (RT-PCR).[10] Every patient with at least one positive HCV test was considered a prevalent case. Patients whose first HCV test was negative were considered incident cases if they tested HCV-positive in subsequent tests. Given that direction of transmission was typically not known, we considered for a given pair (consisting of patients P1 and P2) the HCV status of each patient both as a potential exposure and an outcome. If the HCV status of patient P1 was considered as the outcome, the HCV status of P2 was the exposure variable and vice versa. Biologically, one can interpret the HCV status of the 'exposure patient' as a proxy for the HCV frequency in the transmission network of the outcome patient (see Discussion). In order to take into account the resulting non-independence of the data points, we used in our regression models robust standard errors (based on the clustering of the patients).

We distinguished between the three traditional HIV transmission groups: MSMs,IDUs) and HETs. Given that intravenous drug use is considered to be a far stronger risk factor for HCV transmission than sexual contacts (see Table 1 and reference 4), we considered those HETs and MSMs for which intravenous drug use was reported (but not as the likely route of HIV transmission) as the following separate risk groups: HET-I (individuals who stated having acquired HIV heterosexually but also reported intravenous drug use) and MSM-I (men who stated having acquired HIV by homosexual contact but also reported intravenous drug use).

The impact of phylogenetic status on the incidence of HCV (i.e. the hazard with which initially HCV-negative individuals acquired HCV) was determined using univariable and multivariable Cox proportional hazard models. In these models, the time of the first negative HCV test was chosen as the time-origin for each patient. The proportional hazards assumption was tested by Schoenfeld residuals and it could not be rejected (at $P > 0.05$).

Results

Among the 9748 individuals in the SHCS-DRDB with information on their HCV status, 2768 (28.4%) had at least one positive HCV test and were classified as HCV-prevalent cases, and 208 (2.1%) had a first negative and a subsequent positive HCV test and were classified as incident cases. HCV prevalence differed strongly across risk groups. Specifically, 2038/2147 (94.9%) IDUs, 200/3167 (6.3%) HETs and 190/3568 (5.3%) MSMs had at least one positive HCV test. Further, 318 HETs and 162 MSMs reported previous intravenous drug use and were classified as separate risk groups (HET-I and MSM-I). HET-I and MSM-I individuals exhibited a much larger HCV prevalence than the remaining HETs and MSMs (71.7% for HET-I vs 6.3% for HET, and 27.2% for MSM-I vs 5.3% for MSM), which is consistent with the fact that intravenous drug use is a much more efficient transmission route for HCV than sexual contact. Other risk-groups (perinatal, blood transfusion etc.) contributed 386 patients (4%; 318 HCV-negative, 68 HCV-positive), who were excluded from further analysis. The baseline characteristics of the remaining study population (9362 patients) are summarized in Table 1.

We found that HCV status was strongly associated with HIV subtype (Table 1). Overall, >90% of HCV cases occurred in individuals with HIV-1 subtype B, and hence we restricted the phylogenetic part of the analysis to this subtype. Nevertheless it should be noted that, at least in Switzerland, a non-B subtype HIV-1 infection implies a lower risk of an HCV coinfection than a subtype B HIV infection [adjusted OR = 0.5 (0.4, 0.6), see Table 1]. The main reason for the difference between subtypes is their composition in terms of transmission groups. As we have shown previously,[14] all three main transmission groups (HETs, MSMs and IDUs) are well represented among individuals with a HIV-1 subtype B infection. By contrast, infections with non-B HIV-1 are limited almost exclusively to HETs.[12,20,21] We found a similar difference in the distribution of transmission groups for B and non-B HIV-1 subtypes among individuals included in this study (see Table S1, available as Supplementary data at *IJE* online). Thus the major drivers of HCV transmission (IDU in the past and MSM in the present) are missing from non-B subtypes, explaining the difference in HCV prevalence. This indicates that the HCV epidemic in Switzerland is more closely connected to the transmission networks of HIV-1 subtype B than to those of non-B subtypes.

For subtype-B HIV infections, we considered the clustering of Swiss HCV cases on the HIV phylogeny derived from 7644 Swiss and 36 227 non-Swiss *pol* sequences (see Methods and Figure 1). On this phylogeny, we could identify 1555 potential HIV-1 transmission pairs, for which the HCV status was known for both members. In 907, 303 and 345 of these pairs none, one and both patients were HCV–infected, respectively. This implies that for a patient in a given transmission pair, the odds of having a HCV coinfection was increased by an OR of 13.6 [95% CI (10.5, 17.6)] if the other patient in the pair had an HCV coinfection as well. This extremely high OR was largely due to the tendency of IDUs to cluster with other IDUs.[14] Since most HCV cases occurred in IDUs, this clustering of IDUs with each other resulted in a strong confounding of the OR by patient risk group. However, a strong association between the HCV status of the patients in a transmission pair persisted, even if risk groups were considered separately. Specifically, the ORs were 5.4 (2.9, 10.3) for

Table 1. Baseline characteristics of the study population consisting of individuals from the SHCS-DRDB with at least one HCV test available

Characteristic	HCV- (%)	HCV+ (%)	Total	OR (95% CI) for HCV+ in univariate model	OR (95% CI) for HCV+ in multivariate model
Transmission group					
HET	2967 (93.7)	200 (6.3)	3167	Baseline	Baseline
HET-I	90 (28.3)	228 (71.7)	318	37.6 (28.3, 49.9)	27.8 (20.6, 37.5)
IDU	109 (5.1)	2038 (94.9)	2147	277.4 (218.2, 352.6)	179.1 (138.7, 231.1)
MSM	3378 (94.7)	190 (5.3)	3568	0.8 (0.7, 1)	0.6 (0.5, 0.7)
MSM-I	118 (72.8)	44 (27.2)	162	5.5 (3.8, 8)	4 (2.7, 6.1)
HIV subtype					
B	4748 (65.5)	2506 (34.5)	7254	Baseline	Baseline
Non-B	1914 (90.8)	194 (9.2)	2108	0.2 (0.2, 0.2)	0.5 (0.4, 0.6)
Sex					
Male	4922 (73.5)	1777 (26.5)	6699	Baseline	Baseline
Female	1740 (65.3)	923 (34.7)	2663	1.5 (1.3, 1.6)	0.9 (0.7, 1.1)
Centre					
Zurich	2755 (72.6)	1041 (27.4)	3796	Baseline	Baseline
Basel	695 (70.6)	289 (29.4)	984	1.1 (0.9, 1.3)	1 (0.8, 1.3)
Bern	782 (67.1)	383 (32.9)	1165	1.3 (1.1, 1.5)	1 (0.8, 1.4)
Geneva	997 (76.9)	300 (23.1)	1297	0.8 (0.7, 0.9)	0.7 (0.6, 1)
Lausanne	935 (72.1)	361 (27.9)	1296	1 (0.9, 1.2)	1.3 (1, 1.6)
Lugano	167 (60.7)	108 (39.3)	275	1.7 (1.3, 2.2)	1.7 (1, 2.6)
St Gallen	331 (60.3)	218 (39.7)	549	1.7 (1.4, 2.1)	1.3 (0.9, 1.8)
Registration year					
–1989	257 (40.1)	384 (59.9)	641	Baseline	Baseline
1990–94	790 (53.6)	685 (46.4)	1475	0.6 (0.5, 0.7)	0.8 (0.6, 1.2)
1995–99	1789 (66)	921 (34)	2710	0.3 (0.3, 0.4)	0.6 (0.4, 0.8)
2000–04	1563 (78)	441 (22)	2004	0.2 (0.2, 0.2)	0.6 (0.4, 0.8)
2005–	2263 (89.4)	269 (10.6)	2532	0.1 (0.1, 0.1)	0.3 (0.2, 0.5)
Age at registration (years)					
–29	1573 (60.9)	1008 (39.1)	2581	Baseline	Baseline
30–39	2673 (67.9)	1264 (32.1)	3937	0.7 (0.7, 0.8)	1.2 (1, 1.5)
40–49	1534 (81.1)	358 (18.9)	1892	0.4 (0.3, 0.4)	1.2 (0.9, 1.5)
50–59	600 (91.7)	54 (8.3)	654	0.1 (0.1, 0.2)	1.1 (0.8, 1.6)
60–	282 (94.6)	16 (5.4)	298	0.1 (0.1, 0.1)	0.8 (0.5, 1.5)

HETs, 2.7 (1.3, 5.5) for IDUs, 3.1 (1.4, 7.0) for MSMs, 2.1 (0.9, 5.1) for HET-I and 4.5 (1.2, 16.3) for MSM-I. In terms of HCV prevalence, this implies for example that among MSMs that were paired with HCV-negative patients, the prevalence was 4.1% but increased to 11.8% for MSMs that were paired with HCV-positive patients.

When adjusted for sex, age, risk group, year of registration in the SHCS, and treatment center (= proxy for geographical location), in a multivariable analysis, we also found that patients in transmission pairs were much more likely to be HCV-positive if their partner in the pair was HCV-positive [OR 3.2 (2.2, 4.7), see table 2]. Thus, we observed a strong clustering of prevalent HCV cases on the HIV phylogeny even after controlling for the most important demographic confounders.

The extent to which the HCV status of neighbouring patients could predict the risk of an HCV infection depended on the strength of phylogenetic linkage: if restricted to pairs with high local support values on the HIV phylogeny, the degree of HCV clustering became even stronger. Specifically, if the analysis was restricted to pairs with Shimodaira-Hasegawa-support values[22] exceeding 0.7, 0.9, 0.95 and 0.99, we found in the multivariable analysis ORs of 3.4 (2.2, 5.3), 3.4 (1.9, 6.2), 4.0 (1.8, 9.2) and 4.7 (0.97, 22.4), respectively. On the other hand, we extended the classification into high- and low-risk patients beyond transmission pairs. For these patients phylogenetic linkage was weaker than for transmission pairs, but still resulted in a strong predictor of HCV infection [OR 2.1(1.7, 2.6), see supplementary material available as Supplementary data at *IJE* online).

For incident HCV cases, we found that those HCV-negative HIV patients that belonged to a transmission-pair in which the other patient was HCV-positive had a higher

Table 2. Multivariable logistic regression model for HCV prevalence in patients included in HIV transmission pairs (odds ratios are adjusted for all variables listed)

Patients	No. (%)	Odds ratio (95% CI)	*P*-value
HCV Status of Partner in Pair			
HCV-neg	2,047 (67.9)	Baseline	
HCV-pos	966 (32.1)	3.2 (2.2, 4.7)	<0.001
transmission group			
HET	651 (21.6)	Baseline	
HET-I	119 (4)	27.3 (15.6, 47.7)	<0.001
IDU	781 (25.9)	180.2 (112.5, 288.7)	<0.001
MSM	1,387 (46)	0.9 (0.6, 1.5)	0.764
MSM-I	75 (2.5)	4.2 (2.1, 8.6)	<0.001
Sex			
male	2,365 (78.5)	Baseline	
female	648 (21.5)	1.1 (0.7, 1.6)	0.817
Center			
Zurich	1,338 (44.4)	Baseline	
Basel	326 (10.8)	0.9 (0.5, 1.6)	0.694
Bern	376 (12.5)	1.1 (0.7, 1.7)	0.608
Geneva	334 (11.1)	1 (0.7, 1.6)	0.854
Lausanne	355 (11.8)	1.4 (0.8, 2.2)	0.209
Lugano	74 (2.5)	1.2 (0.5, 2.8)	0.746
St. Gallen	210 (7)	1.2 (0.7, 2.1)	0.53
Registration Year			
–1989	208 (6.9)	Baseline	
1990–1994	462 (15.3)	0.8 (0.4, 1.6)	0.583
1995–1999	880 (29.2)	0.6 (0.3, 1.2)	0.154
2000–2004	632 (21)	0.4 (0.2, 0.8)	0.013
2005–	831 (27.6)	0.3 (0.2, 0.6)	<0.001
age at registration			
–29	831 (27.6)	Baseline	
30–39	1,277 (42.4)	1.3 (0.9, 2)	0.117
40–49	636 (21.1)	1.1 (0.7, 1.8)	0.728
50–59	183 (6.1)	1 (0.5, 2.1)	0.978
60–	86 (2.9)	1.7 (0.6, 4.6)	0.281

rate of acquiring HCV than those paired with an HCV-negative partner (Figure 2). Specifically, we found a hazard ratio (HR) of 2.5 (1.5, 4.2) in a univariable Cox proportional hazards model. In the corresponding multivariable analysis adjusted for risk and calendar year, the corresponding HR was 2.1 (1.1, 3.8) (Table 3). The covariates included in this multivariable model differed for the following reasons from the ones included in the analysis of prevalent cases (Table 2). First, it has been shown that the incidence of HCV dramatically increased over the past year for MSMs but decreased for IDUs.[10] Therefore we included calendar year as a separate variable for MSMs and non-MSMs (indeed, Table 3 shows that the hazard for an HCV infection decreased over calendar time for non-MSMs but substantially increased for MSMs). Secondly, we had to reduce the number of variables in the model because only 75 incident HCV cases occurred in transmission

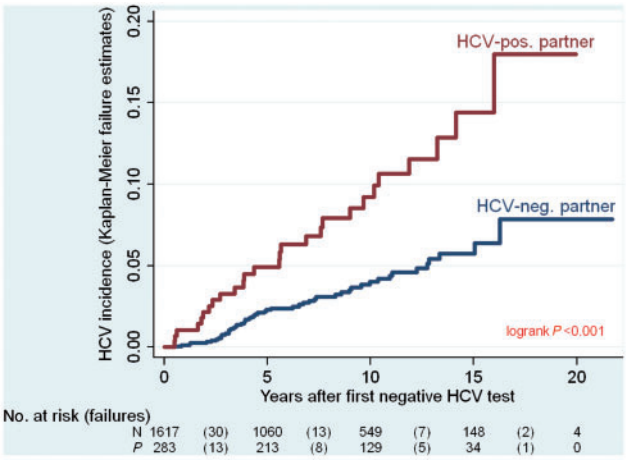

Figure 2. Kaplan_Meyer estimates for HCV incidence in patients paired with HCV-positive (red) and HCV-negative patients (blue) on the HIV phylogeny. In the risk-table below the *x*-axis, N refers to individuals with an HCV-negative partner and *P* to individuals with an HCV-positive partner.

Table 3. Multivariable Cox proportional hazard model for incident HCV cases (odds ratios are adjusted for all variables listed). The time of the first negative HCV-test was chosen as the time-origin for each patient. The proportional hazards assumption was tested by Schoenfeld residuals and it could not be rejected ($P > 0.5$)

Patient	At risk, No. (%)	No. of incident cases (%)	Hazard ratio (95%CI)	*P*
HCV Status of Partner in Pair				
HCV-neg	1,567 (85.2)	49 (65.3)	Baseline	
HCV-pos	272 (14.8)	26 (34.7)	2.1 (1.1, 3.8)	0.018
transmission group				
HET	528 (28.7)	4 (5.3)	Baseline	
HET-I	28 (1.5)	4 (5.3)	16.2 (3.9, 67.3)	<0.001
IDU	47 (2.6)	22 (29.3)	49.5 (16.1, 151.7)	<0.001
MSM	1,182 (64.3)	40 (53.3)	1.3 (0.3, 4.8)	0.719
MSM-I	54 (2.9)	5 (6.7)	13.3 (3.6, 49.5)	<0.001
Calendar Year				
for MSMs			1.2 (1.1, 1.3)	0.002
for non-MSMs			0.9 (0.8, 1.0)	0.073

pairs. For this reason, we omitted the variables 'age', 'sex' and 'centre', which only had a weak impact on prevalent cases, and treated 'calendar year' as a continuous variable. We found however almost the same impact of the HCV status in the transmission-pair partner when including the same covariates as for prevalent cases [HR 2.2 (1.2, 4.1)]. Finally, we found a comparable HR for the impact of HCV exposure if we restricted the multivariable analysis to MSMs, at 2.29 (0.9, 5.4). This suggests that the observed doubling of HCV incidence in individuals clustering with HCV-coinfected partners on the HIV phylogeny was, at least in part, mediated through sexual transmission of HCV.

Finally, we considered the distribution of HCV genotypes on the HIV phylogeny. HCV genotype information was available for 1302 patients infected with HIV-1 subtype B in the SHCS-DRDB. This resulted in 99 transmission pairs for which HCV genotype information was available for both members. The most frequent HCV genotypes were 1A ($n = 370$), 3A ($n = 361$), 1B ($n = 192$), and 4C/4D ($n = 105$). Taking this genotype information into account, we found pairings with consistent genotypes in 39 out of 99 pairs. We assessed whether this pattern was stronger than expected by chance by comparing it with the genotype concordance after randomizing the genotypes of the 99 transmission pairs that had genotype information for both members (10^4 replicates). This randomization test revealed that the observed degree of genotype clustering exceeded the genotype concordance expected by chance ($P = 0.007$). Across randomizations, the median (95% CI) number of pairs with consistent genotypes was 28 (20, 36); thus the observed number of pairs with consistent genotypes exceeded the expectation by a factor of 1.4 (1.1, 2.0).

Despite the fact that genotypes clustered more strongly than expected by chance (with $P = 0.007$), the overall strength of this clustering was relatively weak. This indicates that the clustering of HCV cases on the HIV phylogeny was caused by both direct and indirect mechanisms (see Discussion). However, the weak genotype clustering might also reflect the fact that individual patients have experienced multiple, subsequent HCV infections and might therefore have switched their HCV genotype. Of 217 patients with more than one HCV genotype measurement, 13 experienced a switch in HCV genotype, which corresponded to a genotype switching rate of 1/49.6 patient-years. This might be a strong underestimate of the frequency of superseding HCV infections that occurred in the 1980s/90s (when most IDUs acquired their HCV infection) because, on the one hand, patients reduce their risk behaviour once an HIV infection is diagnosed[25] and, on the other hand, the risk of HCV transmissions among IDUs in Switzerland has strongly decreased over the past years as a result of needle-exchange programmes. This hypothesis is supported by the fact that HCV superinfections have been shown to be frequent in other settings (without IDU-targeted prevention efforts comparable to Switzerland).[26] Thus the relatively weak clustering of HCV genotypes as compared with the clustering of HCV cases might reflect a very dynamic early HCV epidemic with frequent HCV subtype substitutions.

Discussion

Even though prevention efforts in the past two decades have been successful in some high-risk populations,[10] the spread of HCV is still a major public health concern.[5] The recent spread of HCV among HIV-infected MSMs is

especially worrisome as it underlines the dynamic and adaptable nature of the HCV epidemic. Whereas the decrease of HCV transmission among IDUs can be attributed to the public health measures addressed to this transmission group (needle-exchange programmes, methadone and heroin-substitution programmes etc.),[10] the reasons underlying the increase of HCV among MSMs are much less certain. The fact that incident HCV infections occur preferentially in MSMs with high-risk sexual behaviour,[9] the association of HCV with other sexually transmitted infections (STIs)[10] and the recent decrease in condom use[11] suggest however that the rise of HCV among MSMs may be due to increased risk-taking in this transmission group. Therefore it is crucial to understand the interaction between HIV and HCV and to be able to identify high-risk populations for the spread of HCV beyond the obvious candidates (such as IDUs).

Our finding that HCV-cases cluster on the HIV-phylogeny, even after taking the most important demographic variables into account, can be interpreted in two ways. The direct interpretation explains the clustering by the fact that HIV and HCV directly share transmission routes. A clustering of two patients on the HIV phylogeny implies a proximity on the contact network (defined by those transmission routes), and hence also on the contact network on which HCV can spread. In other words, two individuals who cluster on the HIV phylogeny are also more likely to belong to the same HCV transmission chain. The indirect interpretation explains the clustering by assortative mixing of high-risk subpopulations. It is a general feature of the molecular epidemiology of HIV that patients cluster preferentially with other patients of the same demographic and social strata.[14,20] Accordingly, if some classes are more prone to be infected with HCV, then HCV cases and hence also their neighbours on the HIV phylogeny will more likely belong to those classes. The multivariable analyses (Tables 2 and 3) partially adjust for this effect, but other demographic variables, such as belonging to a local high-risk population, may cause an additional clustering of HCV cases on the HIV phylogeny. Our finding that individual HCV genotypes clustered more strongly than expected by chance (with $P = 0.007$) but that the magnitude of this effect was weak, indicates that both the direct and the indirect mechanisms are responsible for the clustering of HCV cases on the HIV phylogeny.

Whatever the underlying reasons for the clustering of HCV on the HIV phylogeny, our results indicate that the transmission networks of HIV and HCV are correlated and overlap even beyond the degree that can be expected by demographic variables such as riskgroup (especially IDUs), geography, sex and age. Thus, our analysis shows that the location of an HIV-infected patient on the HIV phylogeny can serve as an indicator for the risk of an HCV coinfection: Patients whose HI virus is closely related to the HI virus of HIV/HCV coinfected patients have a higher risk of carrying HCV themselves and, if they are HCV-negative, they have a higher risk of becoming infected with HCV. Accordingly, such patients could constitute target groups where intensified testing and counselling are particularly important. Alternatively, the phylogenetic information could be integrated with other information such as high-risk sexual practices[9] (information not available in our dataset) to derive a combined risk assessment score.

This study has several limitations. First, the method to detect clustering of HCV cases is only indirect. Instead of directly observing clusters of Swiss HCV cases on a phylogeny from HCV sequences, we consider the distribution of HCV sequences on the HIV phylogeny. As outlined above, this type of clustering is consistent both with shared transmission networks and with a clustering of risk factors (for HCV acquisition) on the HIV phylogeny. A direct quantification of the fraction of domestically transmitted HCV infections would require sequencing HCV from infected patients and goes therefore beyond the scope of this study. Second, as in all similar phylogenetic studies, our results potentially depend on how phylogenetic clusters are defined; here we have focused on monophyletic pairs of patients (irrespective of the statistical support for the clustering). However, we found similar results if we restricted the analysis to pairs with high support values (i.e. where the clustering was strongly supported by the phylogeny, see Results), and if we extended the analysis beyond pairs (see supplementary material available as Supplementary data at *IJE* online). Finally, these results stem from the particular epidemiological setting of the SHCS, and similar analyses in other cohorts are required to assess the generalizability of these findings.

Our results have several implications for public health. The strong clustering of Swiss HCV that we found for all transmission groups implies that the location of an HIV-1 infected patient on the HIV phylogeny can be used as a measure of that patient's risk of acquiring an HCV infection. Furthermore, the clustering indicates an important role of domestic HCV transmission in the Swiss HCV epidemic, which in turn suggests that preventive interventions can limit the spread of HCV even if they are locally limited to Switzerland. The fact that this pattern was also observed for MSM suggests that domestic transmission occurs for sexually transmitted HCV as well. Specifically, we found that MSMs paired with HCV-positive partners have a more than 2-fold higher HR of acquiring HCV themselves, highlighting the importance of safe sex practices in HCV-discordant MSM couples and in sex with unknown

partners even if HIV is suppressed by highly active antiretroviral therapy (HAART).

Supplementary Data

Supplementary material is available as Supplementary data at *IJE* online.

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References

1. World Health Organization. *WHO Fact Sheet: Hepatitis C*. Geneva: WHO, 2013.
2. Rosen HR. Chronic hepatitis C infection. *N Engl J Med* 2011;**364**:2429–38.
3. Freeman A. Estimating progression to cirrhosis in chronic hepatitis C virus infection. *Hepatology* 2001;**34**:809–16.
4. Vickerman P, Hickman M. The effect of epidemiological setting on the impact of harm reduction targeting injecting drug users: In: *Harm reduction: evidence, impacts and challenges*. Lisbon: European Monitoring Centre for Drugs and Drug Addiction, 2010.
5. Theodore Sy MMJ. Epidemiology of hepatitis C virus (HCV) infection. *Int J Med Sci* 2006;**3**:41.
6. Danta M, Rodger AJ. Transmission of HCV in HIV-positive populations. *Curr Opin HIV AIDS* 2011;**6**:451–58.
7. van de Laar TJW, van der Bij AK, Prins M *et al*. Increase in HCV incidence among men who have sex with men in Amsterdam most likely caused by sexual transmission. *J Infect Dis* 2007;**196**:230–38.
8. Götz HM, van Doornum G, Niesters HG, Hollander den JG, Thio HB, de Zwart O. A cluster of acute hepatitis C virus infection among men who have sex with men – results from contact tracing and public health implications. *AIDS* 2005;**19**:969–74.
9. Vogel M, Boesecke C, Rockstroh JK. Acute hepatitis C infection in HIV-positive patients. *Curr Opin Infect Dis* 2011;**24**:1–6.
10. Wandeler G, Gsponer T, Bregenzer A *et al*. Hepatitis C virus infections in the Swiss HIV Cohort Study: A rapidly evolving epidemic. *Clin Infect Dis* 2012;**55**:1408–16.
11. Hasse B, Ledergerber B, Hirschel B *et al*. Frequency and determinants of unprotected sex among HIV-infected persons: the Swiss HIV cohort study. *Clin Infect Dis* 2010;**51**:1314–22.
12. Swiss HIV Cohort Study, Schoeni-Affolter F, Ledergerber B *et al*. Cohort profile: the Swiss HIV Cohort study. *Int J Epidemiol* 2010;**39**:1179–89.
13. Paraskevis D, Pybus O, Magiorkinis G *et al*. Tracing the HIV-1 subtype B mobility in Europe: a phylogeographic approach. *Retrovirology* 2009;**6**:49.
14. Kouyos RD, Wyl von V, Yerly S *et al*. Molecular epidemiology reveals long-term changes in HIV type 1 subtype B transmission in Switzerland. *J Infect Dis* 2010;**201**:1488–97.
15. Worobey M, Gemmel M, Teuwen DE *et al*. Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960. *Nature* 2008;**455**:661–64.
16. Leigh Brown AJ, Lycett SJ, Weinert L *et al*. Transmission network parameters estimated from HIV sequences for a nationwide epidemic. *J Infect Dis* 2011;**204**:1463–69.
17. Prosperi MCF, Ciccozzi M, Fanti I *et al*. A novel methodology for large-scale phylogeny partition. *Nat Commun* 2011;**2**:321.
18. Gifford RJ, de Oliveira T, Rambaut A *et al*. Phylogenetic surveillance of viral genetic diversity and the evolving molecular epidemiology of human immunodeficiency virus type 1. *J Virol* 2007;**81**:13050–56.
19. Hué S, Hassan AS, Nabwera H *et al*. HIV type 1 in a rural coastal town in Kenya shows multiple introductions with many

subtypes and much recombination. *AIDS Res Hum Retroviruses* 2012;**28**:220–24.

20. Wyl von V, Kouyos RD, Yerly S *et al*. The role of migration and domestic transmission in the spread of HIV-1 non-B subtypes in Switzerland. *J Infect Dis* 2011;**204**:1095–103.
21. Wyl von V, Yerly S, Böni J *et al*. Emergence of HIV-1 drug resistance in previously untreated patients initiating combination antiretroviral treatment: a comparison of different regimen types. *Arch Intern Med* 2007;**167**:1782–90.
22. Price MN, Dehal PS, Arkin AP. FastTree 2 – approximately maximum-likelihood trees for large alignments. *PLoS One* 2010;**5**:e9490.
23. Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 2006;**22**:2688–90.
24. Liu K, Linder CR, Warnow T. RAxML and FastTree. Comparing two methods for large-scale maximum likelihood phylogeny estimation. *PLoS One* 2011;**6:**e27731.
25. Deren S, Beardsley M, Coyle S, Singer M. HIV serostatus and risk behaviors in a multisite sample of drug users. *J Psychoactive Drugs* 1998;**30**:239–45.
26. Herring BL, Page-Shafer K, Tobler LH, Delwart EL. Frequent hepatitis C virus superinfection in injection drug users. *J Infect Dis* 2004;**190**:1396–403.